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Investigating the Effects of the BDNF Val66Met Polymorphism and Physical Activity on Executive Functioning, Short-term Memory, Long-term Memory and Learning in Older Adults.

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Statement of Sources

I declare that this report is my own original work and that the contributions of others have been duly acknowledged.

Signed:

Melissa Jay Heather

Date: 27/10/2017

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Investigating the Effects of the BDNF Val66Met Polymorphism and Physical Activity on Executive Functioning, Short-term Memory, Long-term Memory and Learning in Older Adults.

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The decline of cognitive abilities with age is subject to much variability, and the factors that influence this are yet to be fully understood. This study aimed to explore the combined influence of physical activity (PA), and the BDNF gene on cognitive performance in older adults. Participants consisted of 140 female and 61 male (N = 201), healthy older adults, aged 53 – 81 years (*M* = 64.2, *SD* = 6.63). BDNF genotype was ascertained via genetic testing, and participants were categorized as either Val carriers or Met carriers. Participants were also grouped into high or low PA, as assessed with the self-report Incidental and Planned Exercise Questionnaire (IPEQ-WA). Cognitive performance was assessed via neuropsychological tests of executive functioning, short-term memory, long-term memory and learning. It was hypothesised that level of PA and BDNF genotype would have main effects on cognitive performance and that there would be an interaction between level of PA and BDNF genotype on cognitive outcome measures. However, contrary to our hypotheses, no significant main effects or interaction effects were observed between level of PA and the BDNF gene on any of the cognitive performance measures. These results suggest that, in a group of healthy older adults, level of PA and BDNF genotype are unrelated to cognitive performance.

A natural consequence of the human aging process is a decline in cognitive abilities (Salthouse, 2009). Behavioural research has identified clear patterns of reduction in various cognitive functions with age, particularly areas of memory and executive function (Hedden & Gabrieli, 2004). However, cognitive decline is subject to high levels of variability and many studies are now investigating what contributes to individual susceptibility, examining elements such as environmental and lifestyle influences or genetic factors (Erikson et al., 2008; Fratiglioni, Paillard-Borg & Winblad, 2004). Physical Activity (PA) is a lifestyle factor linked to the maintenance and improvement of neurocognitive health (Stillman, Cohen, Lehman & Erikson, 2016). Higher levels of physical activity (activities in day-to-day life that involve moving the body and engaging the muscles) are associated with reduced risk of chronic diseases and more efficient cognitive function (Bherer, Erikson & Liu-Ambrose, 2013). There is compelling research that supports PA as a moderator of cognitive decline, however individual differences are still evident (Bherer et al., 2013). The genetic and neurobiological components that underpin this process may provide further understanding.

The BDNF gene controls the secretion and distribution of brain-derived neurotrophic factor (BDNF). BDNF is a neurotrophin found in the brain that plays a pivotal role in neural circuit development and growth, synapse formation and plasticity as well as memory and higher cognitive functions (Park & Poo, 2013). Recent genetic studies have identified a polymorphism in the human BDNF gene, known as Val66Met, which results in different versions of the gene that produce different responses in the regulation of BDNF (Park & Poo, 2013). Furthermore, genetic association studies have demonstrated that Val66Met plays a role in the variability of cognitive performance with aging (Miyajima, 2008).

Research has begun to examine how environmental influences, such as PA, can impact genetic predispositions, like BDNF Val66Met, in cognitive outcomes. Neurobiological studies have demonstrated that PA increases levels of neurotrophins particularly BDNF (Knaepen, Goekint, Heyman & Meeusen, 2010). Recently, studies have begun to examine the interaction between the BDNF Val66Met polymorphism and engagement in PA and their effect on cognitive performance in the domains of working memory, episodic memory and executive function (Canivet et al, 2015; Erikson et al., 2013; Thibeau, McFall, Anstey, Wiebe & Dixon, 2016). The present study seeks to expand the understanding of how the BDNF gene interacts with the association between PA and cognitive performance in an older population.

Physical Activity and Cognitive Functioning

The relationship been PA and cognitive performance has been demonstrated in multiple domains of experimental research. In a meta-analysis of 15 observational studies investigating the association between PA and cognitive function in older adults, Sofi et a., (2010) found a significantly reduced risk of cognitive decline for participants engaged in PA. Across the 15 studies 33,816 non-demented subjects (Mean ages > 55) were observed over a 1–12 year period, and assessments of PA were obtained through self-report or questionnaires and cognitive performance was examined predominately through the mini-mental state exam (MMSE). The authors found that even modest levels of PA can have beneficial effects on cognition with the meta-analysis revealing a 38% reduced risk of cognitive decline for participants with a high level of PA compared to sedentary participants and, similarly, a 35% for participants performing low to moderate levels of PA (Sofi et a., 2010).

Clinical intervention research has also extensively assessed the association

between level of PA and cognitive outcomes. One meta-analysis looked at eighteen intervention studies examining the effect of fitness training on the cognitive performance of healthy but sedentary older adults (Colcombe et al., 2003). They found that, compared to controls, exercisers showed significantly improved results of moderate effect size in the cognitive domains of executive function, cognitive control, visuospatial processing and a significant increase of small effect for reaction time (Colcombe et al., 2003). In another review of epidemiological studies that examined modifiable factors associated with cognitive decline and dementia, 24 studies linking PA to cognitive outcomes were assessed and of these 21 (87.5%) found a positive relationship between PA and cognitive performance (Beydoun, Beydoun, Gamaldo, Teel, Zonderman and Wang, 2014). Additionally, this review conducted a meta-analysis on 31 studies examining Alzheimer's disease (AD) and found decreased PA to be a strong predictor of the disease, with lower vs higher levels of PA having a population attributable risk of 31.9% (Beydoun et al., 2014).

Animal studies have also demonstrated the benefits of PA on cognitive function, such as; better long-term and short-term memory in rats exposed to a swimming regime compared to non-exercised controls (Radak et al., 2001); improved object recognition memory for rats assigned to a rodent treadmill, running condition compared to rats in sedentary condition (Bechara & Kelly, 2013); and female cynomolgus monkeys, taught to run on treadmills for an hour a day, were significantly faster at learning the Wisconsin General Test Apparatus (WGTA; designed to test learning in primates) than controls (Rhyu et al., 2010). In addition, animal studies have enabled the research, through more invasive procedures than are possible with human subjects, to reveal the cellular and molecular mechanisms that occur with increased PA that impact the neurological structure of cognition (Kramer

and Erikson, 2007).

The BDNF Val66Met Polymorphism: General Characteristics and Function

BDNF is one of a small number of secreted proteins that have been identified as regulating neural circuit development and function that continues across the lifespan (Poo, 2001). BDNF is initially produced as a precursor, proBDNF and then converted to mature BDNF intracellularly, both versions playing different cellular functions (Yoshida, Ishikawa, Iyo & Hashimoto, 2012). The cellular actions of BDNF occur through binding with one of its two receptors, mature BDNF binds to the high affinity receptor TrkB and proBDNF binds to the low-affinity receptor p75NTR, which often show opposing consequences (Reichardt, 2006). For example, the mature BDNF TrkB receptor facilitates neural survival and hippocampal long-term potentiation (LTP; activity-dependent strengthening of neurons in the hippocampus) while the proBDNF p75NTR receptor, facilitates apoptosis (cell death) and hippocampal long-term depression (LTD; activity-dependent reduction in efficacy of neurons) (Reichardt, 2006; Woo, 2005; Zakharenko, 2003). These cellular functions suggest that while mature BDNF plays a role in promoting memory and learning through stronger neural pathways, proBDNF weakens these connections.

BDNF is produced by the BDNF gene which is located at chromosome 11, an individual's genetic instructions come from the matched pairs of chromosomes from their parents and as such there are two versions of a gene (Freberg, 2015). Alternative versions of a particular gene are known as alleles and the different alleles for the BDNF gene are valine (Val) and methione (Met). This variation in the BDNF gene is called a single nucleotide polymorphism (SNP) which results from an amino acid substitution (valine to methione) at codon 66, known as the BDNF Val66Met

polymorphism (Val66Met) (Egan et al., 2003). The genotype for a given individual reflects the alleles they carry and for BDNF they can occur as Val/Val genotype (known as Val homozygous), Val/Met genotype (known as Val/Met heterozygous) or Met/Met genotypes (known as Met homozygous). Generally, research refers to these genotypes as two groups Val carriers (the Val homozygotes) and Met carriers (both Val/Met and Met/Met genotypes) (Egan et al., 2003). The frequencies of these genotypes vary across populations with Asian populations showing the largest percentage of Met carriers (approx. 44%), European/caucasian populations showing a smaller frequency (approx. 20-25%) and African populations have very few Met carriers (approx.0.05%) (Petryshen et al., 2010).

Cellular biology studies have demonstrated how Val66Met impacts the intracellular distribution and activity-dependent secretion of BDNF in neuronal cells (Chen et al., 2004). The presence of the Met allele has been shown to lead to a decrease in the quantity of mature BDNF released. For Met carriers the structure of proBDNF (the initially released precursor form of BDNF) is altered which affects the conversion to mature BDNF and its ability to bind to its receptor TrkB which, as discussed previously, is the process associated with neurogenesis and hippocampal LTP (Brown et al., 2014; Chen et al., 2004; Reichardt, 2006). The less efficient BDNF trafficking may explain the neurocognitive deficits observed in Met carriers, however, these deficits have proven to be inconsistent particularly in the cognitive performance of an aging population.

The BDNF Val66MetPolymorphism and Cognitive Performance

The BDNF Val66Met polymorphism has been implicated in the variation seen in cognitive performance, with Val carriers and Met carriers demonstrating different cognitive outcomes. A neurocognitive physiological study from Egan et al.

(2003) examined the role of Val66Met in hippocampal based synaptic plasticity, learning and memory in a sample of 641 participants (*M* = 35 years). They assessed hippocampal function through; a cognitive assessment of memory (the Wechsler Memory Scale); neuroimaging to assess the activation of the hippocampus during a memory task; and an intracellular neurochemical measure that assessed hippocampal neuronal integrity and synaptic abundance. Egan and colleagues found lower scores in episodic memory, abnormal patterns of activation in the hippocampus and an impairment in the regulated secretion of BDNF for Met carriers. Miyajima et al. (2008) conducted an observational study examining the impact of Val66Met on the cognitive performance of 722 elderly participants and found Met carriers demonstrated lower scores compared to Val carriers on measures of delayed memory, processing speed and general intelligence.

It has been demonstrated that the effects of Val66Met may become more apparent with age. Li et al. (2009) examined the age-dependent effect of the genetic variant on episodic memory in two studies, one of 382 younger (*M* = 25 years) and another of 566 older (*M* = 66 years), healthy participants. They found no significant difference in memory performance in the younger groups between Val and Met genotypes. However, in the older groups, Met carriers recalled significantly less than Val carriers, suggesting the magnitude of the genetic effect may be impacted by the cognitive decline of aging. A longitudinal study from Ghisetta et al. (2014) assessed Val66Met and the processing speed of 376 older participants (*M* = 83.9 years) over a 13-year period with the Digit-Letter (DL) task. This study also demonstrated an effect of aging in that, while scores at the first assessment did not differ significantly across genotype, Met carriers showed a significantly greater decline in processing speed than Val carriers across six assessments over the 13 years.

An opposite pattern of association has also been demonstrated, with Met carriers demonstrating greater performances compared to Val carriers in some cognitive domains, particular those related to executive function. In a study examining task switching, Gajewski, Hengstler, Golka, Falkenstein and Beste (2011) assessed 131 healthy older adults (M = 70.5 years) on speed and variability of reaction time (RT) and error rates. The Val carriers were observed to be generally slower, had greater reaction time variability and higher error rates than Met carriers (Gajewski et al., 2011). The same team of Gajewski, Hengstler, Golka, Falkenstein and Beste (2012) conducted another study examining Val66Met and the Stroop paradigm (a measure of RT when there is interference in identifying a stimuli) in the same cohort of 131 participants. They found that Met carriers performed better than Val carriers, suggesting a greater ability for interference control in elderly Met carriers.

A meta-analysis conducted by Mandelman and Grigorenko (2012) examined studies that looked at the relationship between Val66Met and cognitive outcomes. Across 31 independent samples, comprising of 7095 subjects, the meta-analysis did not establish any significant relationships between Val66Met genotype and measures of general cognitive ability, memory, executive functioning, visual or cognitive fluency (Mandelman & Grigorenko, 2012). Additionally, a recent study, of 360 subjects between the ages of 50-79 years, found no difference in episodic memory performance between Val carriers and Met carriers (Stuart, Summers, Valenzuela & Vickers, 2014).

The Role of BDNF in the Effect of PA on Cognitive Outcomes

Both human and rodent studies have provided strong evidence that the positive effects of PA on the brain and cognition are facilitated by increased levels of

BDNF, neurogenesis and cell proliferation in the hippocampus (an area of the brain known to play a fundamental role in declarative and spatial memory and the decline of cognitive function with aging) (Kramer and Erikson, 2007; Pinel, 2014).

One early animal study revealed significant, strong, positive associations between the level of PA and the level of BDNF expression in the hippocampal regions of rats (Oliff, Berchtold, Isackson & Cotman, 1998). Another study examined whether BDNF mediated the enhanced learning, demonstrated by exercised rats, by chemically blocking BDNF expression in the hippocampus during exercise (Vaynman, Ying & Gomez-Pinilla, 2004). Vaynman et al. (2004) found that the blocking BDNF reduced the learning and recall abilities to the same level as the sedentary control group, additionally, they found that higher levels of learning and recall were correlated with higher levels of BDNF expression. A more recent rodent study examined age-related memory decline and the associated decrease in neurogenesis (promoted by mature BDNF) and increase in apoptosis (promoted by proBDNF) in the hippocampus (Kim et al., 2011). They compared young and old rats and found that exercise improved short term and spatial memory in older rats. Post-mortem tissue examinations confirmed significant increases in mature BDNF after exercise for both young and old rats and an increase in neurogenesis and reduction in apoptosis for the older exercised rats compared to older sedentary rats (Kim et al., 2011). Increased expression of BDNF following PA has also been found in the striatum of rats, an area of the brain known to play a role in learning through reward and reinforcement and in executive functions such as inhibition of control (Marais, Stein & Daniels, 2009).

Neuroimaging studies of the human brain have revealed an association between BDNF and age-related decline in the size of the hippocampus (Erikson et

al., 2010). In non-demented older adults, the hippocampus has been demonstrated to shrink by as much as 1-2% a year and is one possible mechanism for the normal experience of cognitive decline with aging (Raz et al., 2005). Erikson et al. (2010) conducted MRIs on 142 older adults, without dementia, measured blood serum levels of BDNF and assessed spatial memory. They found smaller hippocampal volume, lower levels of serum BDNF and poorer memory performance were associated with increasing age and, after controlling for age, found that lower levels of serum BDNF were associated with poorer memory and smaller hippocampal size (Erikson et al., 2010). An invention study however, found that an increase in physical activity was associated with increases in serum levels of BDNF, increased hippocampal volume and improved memory (Erikson et al., 2011). Erikson and colleagues conducted a randomized controlled trial examining the effect of exercise training on 120 older adults, with an aerobic exercise group (N = 60) and a stretching control group (N = 60). They found that over a one year period, exercise had increased the size of the hippocampus by 2% (counteracting the natural reduction seen with aging) which was associated with increased serum levels of BDNF and improvements in spatial memory (Erikson et al., 2011).

This research demonstrates a clear association between BDNF, exercise and cognitive performance in aging, however an important element to consider in the role of endogenous BDNF is how it is produced. The fact that the BDNF Val66Met polymorphism has been shown to impact the neurotrophin's distribution and cellular functions leads to the question of how an individual's genotype would interact with the effect of PA on age-related cognitive outcomes (Chen et al., 2004).

Interaction between the BDNF Val66Met Polymorphism and Physical Activity

Research has demonstrated that BDNF is associated with both cognitive

functioning and PA however, only four studies were found that have examined the interaction between the BDNF Val66Met polymorphism, PA and the decline of cognitive performance with aging. These studies have been conducted using varying methods and have resulted in conflicting evidence.

One study from Canivet and colleagues (2015) examined the interaction between Val66Met and PA using a single measure of episodic memory (the Delayed Score of the Logical Memory II subtest of the Wechsler Memory Scale), with 205 participants aged 55 and older. To assess PA, they used two questionnaires (the NASA/JSC PA Scale and the Historical Leisure Activity Questionnaire) to categorise participants as active or inactive based on their METs h/week (a unit for describing energy expenditure). They found that Val carriers demonstrated a significant interaction with levels of PA on episodic memory performance with active Val carriers recalling more items than both active and inactive Met carriers (Canivet et al., 2015). Another study from Thibeau and colleagues (2016) of 577 participants (*M* = 70 years), assessed the same gene and environment interaction with executive function. They found that higher levels of everyday physical activity in Val carriers showed a significant association with better executive function performance that was not seen for Met carriers (Thibeau et al., 2016). Both studies concluded that while PA enhances cognitive performance, it is not enough to counteract the deleterious effects for Met carriers and its influence is more beneficial for Val carriers (Canivet et al., 2015; Thibeau et al., 2016).

In contrast, a study from Kim et al. (2011) examined the interaction between BDNF genotypes and PA with cognitive decline and dementia diagnosis using the mini mental state exam (MMSE) in an elderly population (65 and older). PA was measured using a self-rated 4-point scale, ranging from very active to not at all

active, on questions relating to work and leisure activities. Over a two-year period, they found greater physical activity was associated with less cognitive decline and a decreased number of dementia cases diagnosed across genotypes, however this relationship only reached statistical significance for Met carriers. These results suggest that increased levels of physical activity have significantly more positive effect on cognitive health for Met carriers than Val carriers (Kim et al., 2011).

In addition, a study by Erikson et al. (2013) examined the moderation of Val66Met on the effect of PA with a number of cognitive assessments but in a middle-aged population (M = 44.59 years). PA was assessed using a self-report questionnaire about daily living, work and leisure activities, indexing frequency and duration which results in an estimate of weekly kilocalories expended. They found significant positive associations between levels of PA and the cognitive factors of working memory, episodic memory, switching tasks and visuo-spatial memory but only working memory resulted in a significant interaction effect for Val66Met. Met carriers demonstrated significantly poorer performance in working memory than Val carriers at the lower end of PA but the difference disappeared with greater levels of PA (Erikson et al., 2013). The authors of these two studies conclude that, as a result of increasing BDNF levels from greater PA, Met carriers benefit more robustly from exercise than Val carriers, offsetting the genetic vulnerability (Erikson et al., 2013; Kim et al., 2011).

The methodological differences of the preceding four interaction studies outlined make comparing the evidence challenging. Two of these studies have examined the interaction between PA and BDNF Val66Met with only one aspect of cognitive functioning and each in a different domain (episodic memory or executive function) (Canivet et al., 2015; Thibeau et al., 2016). The third study assesses the

interaction with pathological ageing as assessed by the MMSE (Kim et al., 2011). The MMSE has a number of limitations in regards to accurate diagnosis of dementia and cognitive impairment and it is suggested as a tool more reliable at ruling out cognitive decline that definitively diagnosing it (Ismael, Rajii & Shulman, 2010). The fourth study uses a neuropsychological test battery but examined a middle-age population (Erikson et al., 2013). In addition, all four studies use different methods to assess PA although all are self-reported questionnaires.

Rationale, Aims and Hypotheses

The conflicting evidence demonstrates the complexity of the variation seen in cognitive performance with aging and as such it is unlikely that only one factor alone, such as PA or genotype, explain its trajectory. Therefore, it is necessary for research to focus on the interaction between genes and environmental factors to further understand the etiology of cognitive aging. The proposed study will examine level of PA and BNDF Val66Met with a battery neuropsychological tests, assessing the domains of executive functioning, short-term memory and long-term memory and learning, in a healthy population of 50 years and older to further investigate their relationship with cognitive function.

The aim of this observational study is to examine the interaction of two factors that have both been demonstrated to impact cognitive performance, one environmental factor, level of PA, and one genetic factor, the BDNF Val66Met polymorphism. This study proposes that the more efficient trafficking and regulation of BDNF for the Val carriers, in combination with the increase production of BDNF from PA will result in significantly better scores across cognitive measures for active Val carriers, as compared to Met carriers.

1. We hypothesise that as age-related cognitive decline has been demonstrated to be

mitigated by increased levels of PA, there will be a main effect of PA on cognitive performance whereby participants with higher levels of PA, compared to participants with low levels of PA, will demonstrate significantly better performances across the cognitive measures of executive function, working memory and long-term memory.

2. It is also hypothesised that due to the more efficient trafficking and regulation of BDNF for Val carriers over Met carriers, there will be a significant effect of the BDNF Val66Met polymorphism on cognitive outcomes, whereby Met carriers will demonstrate significantly poorer cognitive performances in measures of executive function, working memory and long-term memory.
3. We predict that the cognitive boost from PA will not be enough to counter the deleterious effect for the Met carriers, and therefore there will be a significant interaction between level of PA and BDNF genotype. Specifically, it is predicted that Val carriers demonstrating high levels of PA will perform significantly better than Met carriers with high levels of PA, and Val carriers who reported low levels of PA will score significantly higher than Met carriers who reported low levels of PA, on measures of executive function, working memory and long-term memory.

Method

Participants

Participants were recruited from a larger sample who had consented to take part in the Tasmanian Healthy Brain Project (THBP; Ethics HREC #H11070) (Summers, Saunders, Valenzuela, Summers, Ritchie, Robinson & Vickers, 2013). The THBP is a prospective longitudinal study examining whether late-life education effects age-related cognitive decline and risk of dementia. Participants were initially

screened by the THBP and excluded if they had any history of conditions associated with cognitive impairment (such as dementia, multiple sclerosis, traumatic brain injury or current psychiatric diagnosis (Summers et al., 2013). Dementia was assessed using the Mattis Dementia Rating Scale and anyone scoring below the recommended cut-off of 133 was excluded (Mattis, 1988). The THBP volunteers were notified of the upcoming ancillary study through a regular newsletter they receive as part of the project and asked to participate in this separate study on the relationship between physical activity, genotype and cognitive function.

Participants for the present study consisted of 61 male and 140 female community-residing healthy older adults. The total number of 201 participants were aged between 53 – 81 years, with a total mean age of 64.2 years (*SD* = 6.63).

Materials

The following tests were used as to assess depression, anxiety and general intellectual capacity. Both anxiety and depression have been demonstrated to negatively impact cognitive performance in older persons (Bierman, Comijs, Jonker & Beekman, 2005). An individual's base level of general intellectual functioning is also a factor that will influence performance in cognitive assessments (Mathias, Bowden & Barrett-Woodbridge, 2007).

Hospital Anxiety Depression Scale (HADS). The HADS is a measure of anxiety and depression in both patients and the general population (Zigmond & Snaith, 1983). It contains 14 items, seven questions for anxiety and seven questions for depression, with higher scores representing greater levels of symptom severity. It is considered a valid and reliable measure of the symptoms of anxiety and depression (Bjelland, Dahl, Haug & Neckelmann, 2002).

Wechsler Test of Adult Reading (WTAR). TheWTAR provides a stable and reliable estimate of intellectual capacity (Wechsler, 2001). Participants are required to read and pronounce 50 words with atypical grapheme to phoneme translation which assesses their ability to apply rules of pronunciation with previous learning. Raw score is then converted to a standardised score of estimated full-scale IQ. The WTAR has been shown to have robust accuracy as a measure of verbal general intellectual skill (Whitney, Shepard, Mariner, Mossbarger and Herman, 2010).

Cognitive Test Battery. A standardized battery of neuropsychological tests was administered by the THBP and all participants completed cognitive assessments annually. The full THBP cognitive assessment took approximately four hours to complete and participants were encouraged to take short breaks as needed to avoid fatigue (Summers, et al., 2013). The results of the following assessments were used in this study as a measure of performance in executive functioning, short-term/working memory, long-term memory and learning.

Executive Functioning

The Trail Making Test (TMT). The TMT is a two-part task. In Part A, the subject has to sequentially connect 25 numbered circles, presented randomly on a sheet of paper, without lifting their pencil from the page. Part B requires the subject to do the same but alternate between sequencing random circles with letters or numbers across the page i.e. connecting from 1 to A to 2 to B etc. For this study, Part B was used, as it is considered a reliable and valid measure of executive control abilities. This is scored as the time (in seconds) it takes participants to complete the task, with a better performance being reflected by a shorter time recorded. A higher performance in the TMT B is an indicator of good executive control abilities, primarily task-switching ability (Sanchez-Cubillo et al., 2009).

The Stroop Test (24 item Victoria version). The Stroop Test (24 item Victoria version) is a brief version of the Stroop task which examines response inhibition. It contains 24 items on 3 tasks, the first two of which require participants to name the colour of dots and neutral words printed in different colours and scores are the total time in seconds it takes to complete the task. The third is an interference task where the words are actually names of colours printed in contrasting colours, i.e. name the colour of the word 'blue' printed in red ink. Scores for the interference task are total time in seconds taken to complete it divided by the time it took to complete the dot task. Performance in this task has been demonstrated to negatively correlate with age and it is considered a psychometrically strong measure of response inhibition in an elderly population (Troyer, Leach & Strauss, 2006).

Controlled Oral Word Association Test (COWAT). The COWAT examines executive function by assessing verbal fluency. This is measured by asking participants to name as many words as possible beginning with the same specified letter but excluding proper nouns such as names of people or places. There are three trials, lasting one minute each for three different letters (e.g. F, A, S). The score is the total number of correct words recorded across three trials. It is considered a valid and reliable clinical instrument for neuropsychological assessment (Ruff, Light, Parker & Levin, 1996).

Short-term Memory

The Digit Span Test (DSP). The DSP is a sub-test of the Wechsler Adult Intelligence Scale (WAIS-IV) used for assessing working memory (Wechsler, 1997). The DSP assesses short-term memory span for auditory-verbal information by asking participants to repeat back numbers in correct order and the longest list correctly recalled is recorded. It is a valid and reliable measure of short-term memory.

The Letter-Number Sequencing Test (LNS). The LNS is a sub-test of the Wechsler Adult Intelligence Scale (WAIS-IV) used for assessing working memory (Wechsler, 1997). The LNS assesses the capacity to manipulate verbally presented information in short-term memory by asking the participant to repeat a sequence of randomly presented letters and numbers but in ascending order for the numbers and alphabetically for the letters. The score is the longest list of items correctly recalled.

Long-term Memory and Learning

Rey Auditory Verbal Learning Test (RAVLT). The RAVLT is a measure of ability to encode, consolidate, store and recall verbal memory. The examiner reads out-loud a series of 15 unrelated words and the participant is required to repeat as many as possible back to the examiner. A series of 5 trials is conducted, and the total of words correctly recalled is recorded for each trial. RAVLT total performance score is represented by adding the results of all five trials. This has been demonstrated to be a valid and reliable measure of episodic declarative memory (de Sousa, Malloy-Diniz & Hamdan, 2012). Additionally, verbal learning can be assessed by comparing the scores across the five trials of the RAVLT.

Rey Complex Figure Test (RCFT). The Rey Complex Figure Test (RCFT) – evaluates visuospatial ability and visual memory by asking subjects to reproduce a complicated line drawing, first by copying and then by delayed recall. It is scored according to the presence of a detail and the location of a detail. The recall trial has been found to be a reliable and valid measure of learning and memory for complex visual information in an elderly population (Berry, Allen & Schmitt, 1991).

Physical Activity Level.

Incidental and Planned Exercise Questionnaire (IPEQ-WA) The IPEQ is specifically designed for use in an elderly population and delineates different types

of physical activities common for this age-group, such as housework, gardening, walking, or a scheduled exercise session (Delbaere, Hauer & Lord, 2010). In the IPEQ-WA, respondents estimate their usual weekly PA over the past three months, which has been demonstrated to show greater reliability by allowing for the week-to-week variability in activity which is more likely to occur in an older cohort (Delbaere, Hauer & Lord, 2010). The IPEQ-WA consists of ten questions, with answers on a six-point Likert scale, asking about particular activities over the past 3 months, average times participated in per week and the average number of minutes engaged in the activity (Doma, Speyer, Leicht & Cordier, 2017). In order to establish standardized levels of PA, the individual scores of the IPEQ-WA activities were summed and then converted into a number known as a MET Value, which is an estimated energy cost of human physical activity. The various activities were classified in terms of MET values by using the MET Values Compendium of Physical Activities, which is a widely-accepted tool for use in clinical and research settings to categorise levels of physical activity (Ainsworth et al., 2011). The total number of minutes exercised per week is then able to be converted to a total number of MET minutes per week. The IPEQ-WA has been evaluated as a valid and reliable measure of PA in later-age cohorts with good criterion validity established against objective measures, such as accelerometers (Merom et al., 2014).

Genetic Testing. DNA was collected via saliva sample by THBP using the Oragene DNA self-collection kits (DNA Genotek Inc., 2012). BDNF genotype has been established by the THBP following the method described by Sheikha, Hayden, Kryski, Smith and Singha (2011). All DNA samples were analysed twice to confirm genotype.

Procedure

After approval from the Tasmanian Human Research Ethics Committee (HREC) (H0016623 see Appendix A) the volunteer participants of the THBP were contacted, via their already established preferred mode of communication, by either email or mail out. A participant information sheet was included, along with a consent form for completion if they chose to proceed with the current study (see Appendix B). To ensure maintenance of confidentiality, all communication with participants and handling of their identifiable data was conducted solely by the authorized project manager from THBP, Dr Kimberley Stuart (who was also a member of this PA study team). After completion of the consent form participants were then asked to complete the IPEQ-WA survey, either through an online link to SurveyMonkey attached to their email or by filling out the paper questionnaire. Upon receipt of the completed consent form and IPEQ-WA, the THBP team allocated each participant an alphanumeric code, and extracted the cognitive and genetic information from the THBP, which was then released to the current study.

Statistical Analysis and Data Screening

Participants were categorized into genotype groups based on their BDNF alleles. Participants were designated as either Val carriers, if they possessed only Val alleles (Val/Val homozygotes) or Met carriers if they possessed one or two Met alleles (Val/Met heterozygotes or Met/Met homozygotes). It is recommended that a range of 500 to 1000 MET minutes per week at minimum is necessary to experience the health benefits of PA (United States Department of Health and Human Services, 2008). As such, the present study categorised participants with MET minutes of 1000 or greater as being in the high PA group and participants with MET minutes of less than 1000 as being in the Low PA group.

SPSS version 24 was used to conduct all analyses. Chi-square and *t*-tests were used to compare both the genotype groups and the level of PA groups on demographic factors. Pearson correlations were used to examine the relationship between age and each of the cognitive performance measures, which revealed significant relationships between age and five of our seven cognitive performance measures, Table 1). As a result, ANCOVAs were performed with age as a covariate in the analysis of the cognitive performance measures.

Table 1.

Correlations Between Age and Cognitive Performance Measures

Cognitive Function	Measure	Correlation with Age (*r*)	*p* value
Executive Functioning	TMT B	.379	<.001
	Stroop Interference	.212	.002
	COWAT	-.088	.192
Short-term Memory	Digit Span	-.098	.146
	LNS	-.268	<.001
Long-term Memory and Learning	RAVLT	-.325	<.001
	RCFT	-.317	<.001

Note: Significance at $\alpha = .05$

The chi-square and *t*-tests revealed there were no significant differences between the demographic measures of age, sex, body mass index (BMI; an indicator of healthy weight in relation to height), HADS scores, WTAR score, years of education, months since cognitive assessment, THBP experimental group and BDNF genotypes, as displayed in Table 2.

The *t*-tests revealed a significant difference between PA level and HADS scores, such that scores of anxiety were moderately higher for the low PA group than

the high PA group t (199) = 3.25, p = .001, d = .53 and scores of depression were also moderately higher for the low PA group than the high PA group t (199) = 3.75, p <.001, d = .61. No other significant differences between level of PA and demographic measures were observed, this analysis is presented in Appendix C.

Table 2.

Demographic Data and Analysis of Differences Between BDNF Val66Met Genotypes

	Met Carrier (n = 77)	Val Carrier (n = 124)	t-test/χ^2	p value
Age (years)				
Mean (SD)	63.96 (6.79)	64.35 (6.55)	-.409	.683
Sex				
Males	23 (29.9%)	38 (30.6%)	.013	.908
Females	54 (70.1%)	86 (69.4%)		
BMI				
Mean (SD)	27.21 (5.42)	27.12 (11.33)	.063	.950
HADS Scores				
Anxiety Mean (SD)	4.23 (3.30)	4.71 (3.17)	-1.019	.309
Depression Mean (SD)	2.13 (2.23)	1.98 (2.3)	.439	.661
WTAR FSIQ				
Mean (SD)	112.71 (5.50)	112.64 (5.63)	.095	.924
Total Years of Education	16.51 (2.95)	16.20 (6.55)	.661	.509
Months since Cognitive Tests	18.16 (7.03)	17.16 (7.46)	.939	.349
THBP Experimental Group				
University Study	65 (84.4%)	94 (75.8%)	2.13	.144
Controls	12 (15.6%)	30 (24.2%)		
Level of PA				
Low	25 (32.5%)	25 (20.2%)	3.85	.050
High	52 (67.5%)	99 (79.8%)		

Note: Significance at α = .05

A series of 2 (BDNF val carrier vs met carrier) X 2 (high PA vs low PA) between subjects ANCOVAS were conducted in order to compare performance between BDNF Val66Met genotype and level of PA on each of the seven cognitive tasks. The dependent variables (DV) were the seven cognitive assessment tasks; the TMT B, Stroop interference, COWAT, Digit Span, LNS, RCFT and RAVLT recall. A repeated measures ANCOVA was run to examine any differences between the two IV groups, BDNF genotype and level of PA, across the 5 RAVLT trials with age as a covariate. Significance was determined with an alpha level of .05. ANCOVAS were conducted using the HADS anxiety and depression measures as covariates revealing a significant effect of depression on performance in the TMT B, F (1,194) = 8.02, p =.005, a significant effect of depression on performance in the RAVLT total, F (1,195) = 5.29, p =.02 and a significant effect of anxiety on performance in the STROOP interference F (1,194) = 3.91, p = .049. Therefore, these measures are used as covariates in the final analysis.

Results

The BDNF allele frequencies were examined using the Hardy-Weinberg Equilibrium chi square and based on the estimated expected frequencies of a caucasian population, 75% Val Carriers and 25% Met Carriers (Petryshen et al., 2010). The observed frequencies were Val/Val genotype (n =124, 62%), Val/Met genotype (n = 70, 35%) and Met/Met genotype (n = 7, 3%) which did not differ significantly from the Hardy-Weinberg equilibrium χ^2 (2, N=201) = 3.81, p = 0.149. The frequency for the two genotype groups used for analysis in this study were Val carrier (n = 124, 62%) and Met carrier (n = 77, 38%).

Tests of normality and skewness indicated that scores on all the cognitive measures deviated from normal so logarithmic and square root transformations were

performed to investigate their effect on the data. However, as the transformations did not significantly alter results, and due to the robustness of ANOVA to violations of normality, the untransformed data was analysed (Field, 2013). Windsorizing was used to convert extreme outliers to the nearest non-outlier score (Field, 2013).

Means and standard deviations for all neuropsychological test results, excluding the RAVLT 1 to 5, are presented in Table 3. The mean scores of the RAVLT trials are presented in figure 1, where the learning curve for each of the IV groups is illustrated.

Table 3.

Means and Standard Deviations for Neuropsychological Test Performance by BDNF Genotype and Level of Physical Activity.

	Met Carrier		Val Carrier	
	Low PA	High PA	Low PA	High PA
	n = 25	*n = 52*	*n = 25*	*n = 99*
Executive Functioning				
TMT B[a]	60.91 (21.19)	54.55 (29.34)	50.60 (17.36)	55.73 (22.77)
Stroop Interference	1.81 (.30)	1.81 (.43)	1.81 (.36)	1.84 (.33)
COWAT	51.84 (8.67)	50.96 (11.75)	52.44 (9.97)	52.85 (11.09)
Short-term Memory				
Digit Span	18.72 (3.70)	19.37 (4.08)	19.20 (3.92)	18.57 (3.68)
LNS	11.68 (2.10)	11.88 (2.48)	11.96 (2.88)	11.77 (2.32)
Long-term Memory and Learning				
RAVLT	50.52 (7.09)	52.08 (10.23)	51.64 (10.70)	53.48 (9.26)
RCFT	27.86 (6.53)	29.22 (5.61)	29.20 (4.96)	28.69 (6.21)

Note: [a] A lower score on this test indicates better performance. Figures in parentheses are standard deviations.

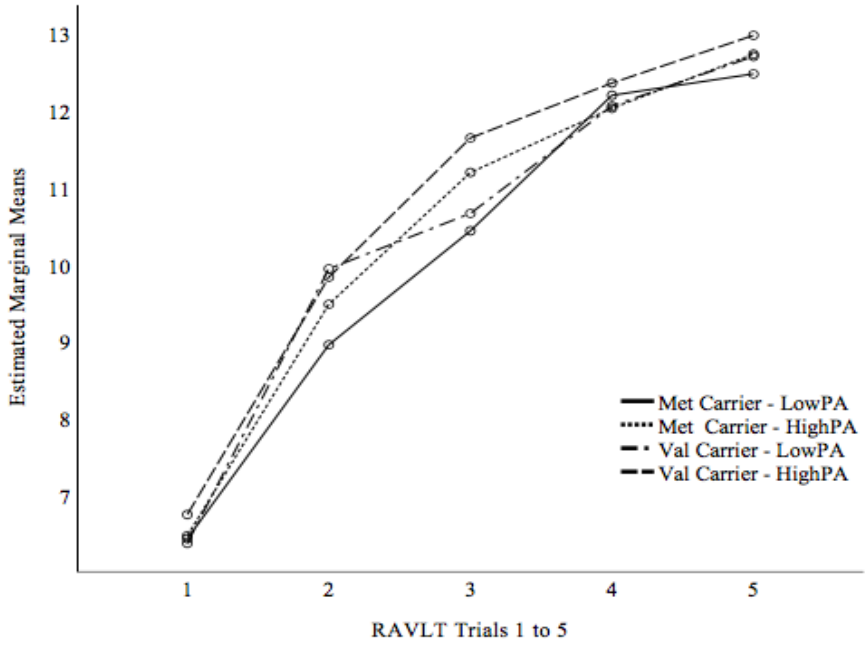

Figure 1. The mean scores for trials one to five of the RAVLT and the learning curve for each of the four independent variables; Met carrier with Low PA, Met carrier with High PA, Val carrier with Low PA and Val carrier with High PA.

Main effects of BDNF Genotype and Physical Activity Level on Cognitive Performance

The 2 Way ANCOVAs revealed no significant main effects of BDNF genotype (Val carrier or Met carrier) on any of the cognitive performance measures of executive functioning, short-term memory or long-term memory, indicating there are no differences in cognitive processing due to BDNF polymorphic variation. There was also no significant main effect of level of PA (Low PA or High PA) on performance in any cognitive domain, with no differences in functioning between PA groups on measures of executive functioning, short-term memory or long-term memory. Contrary to our hypotheses, there were no significant interactions between BDNF Val66Met genotype and level of PA on any measures of cognitive

performance. The repeated measures ANCOVA found no significant difference in performance across the 5 trials of the RAVLT between BDNF genotype F (4,693) = 1.36, p =.25, η_p^2 = .007 or level of PA F (4,693) = 2.43, p = .054, η_p^2 = .012 and no significant interaction F (4,693) = 1.154, p = .33, η_p^2 = .006, following Greenhouse-Geisser adjustments due to violations of sphericity. The ANOVA results are presented in Appendix D.

Discussion

The decline of cognitive abilities with age is subject to much variability and this study aimed to explain some of this variance through the combined influence of one environmental factor, level of PA, and one genetic factor, BDNF genotype. Contrary to our hypotheses, no significant main effects or interaction effects were observed between level of PA and the BDNF Val66Met polymorphism on any of the cognitive performance measures of executive function, short term memory or long-term memory and learning.

Our first hypothesis was that there would be a main effect of PA on cognitive outcomes, whereby participants with higher levels of PA would perform better than participants with low levels of PA. While the significant benefits of physical activity on cognitive performance have been demonstrated in research studies and clinical interventions (Colcombe et al., 2003; Sofi et a., 2010), we found no significant difference in scores between high levels of PA and low levels of PA in the cognitive performance measures of executive function, short-term memory, or long-term memory and learning, and therefore our first hypothesis was not supported.

Our second hypothesis was that there would be a main effect of the BDNF Val66Met polymorphism on cognitive performance, whereby Met carriers (participants with the at-risk allele) would achieve lower scores in the cognitive

measures than Val carriers. Although previous research has shown that the BDNF Val66Met polymorphism is associated with significant differences in cognitive outcomes between genotypes (Egan et al., 2003; Ghisetta et al., 2014; Miyajima et al., 2008), we found cognitive performance did not vary between BDNF genotypes, Val carriers and Met carriers, across all the measures of executive function, short-term memory or long-term memory and learning. This is consistent with the results of Mandelman and Girgorenko (2012) and Stuart et al., (2014) who also found no evidence of significant relationships between cognitive performance and the BDNF Val66Met polymorphism. Therefore, our second hypothesis was not confirmed, but aligns with some previous research, as will be considered further below.

Our third hypothesis was that there would be a significant interaction between BDNF genotype and level of PA, with Val carriers in the High PA group outperforming Met carriers in the High PA group and Val carriers in the Low PA group outperforming Met carriers in the Low PA group in measures of cognitive performance. This is the first study to be conducted examining the interactive effect between level of PA and the BDNF Val66Met polymorphism on cognitive function in an older adult population using a battery of neuropsychological tests, and as such cannot be directly compared against previous findings. However, there was no evidence of any interactions and therefore these results suggest that, in a group of healthy older adults, level of PA and BDNF genotype do not interact in a manner which significantly influences cognitive performance in older adults.

The findings of our study partially align with those from Erickson et al. (2013) who found no significant interaction between BDNF and PA on measures of episodic memory, visuo-spatial memory or task-switching in a longitudinal study of a middle-age population (Mean Age = 44.59 years). However, conflicting with our

results, they did find a significant interaction effect on four assessments of working memory in that higher levels of PA resulted in improved performance for Met carriers only, suggesting that for working memory Met carriers benefit more from PA than Val carriers. Our results also conflict with those of Kim et al. (2011), who found that higher levels of PA were significantly more beneficial for Met carriers, who showed less cognitive decline than Val carriers with increased activity, in a population of 732 community elders (Mean Age = 71.2 years). Their results showed significantly better scores on the MMSE as PA levels increased for participants possessing the Met allele.

It is important to consider how the previous evidence reflects the actual behavioural outcomes for participants and whether the differences are enough to indicate a meaningful difference in cognitive performance. Erickson and colleagues (2013) had a sample size of over 1000 participants and the effects sizes they observed were very small. Three of their working memory measures reported effect sizes partial r^2 that were less than .01 and the fourth had an effect size of partial r^2 = .019. In a guide for meaningful effect sizes in clinical terms, Ferguson suggests that for clinical relevance, partial r^2 of at least .04 is needed, which suggests that whilst significant, Erickson et al's findings may not be substantive enough to indicate practical importance. Additionally, Kim et al. (2011) reported odds ratios which ranged from 1.50 – 1.60 for cognitive decline in each of their logistic regression models for which Ferguson (2009) advises at least 2.0 to be meaningful. As cognitive decline was measured by a reduced score in the MMSE from baseline to the follow-up assessment the odds ratios suggests that while scores were significantly different they may not be practically meaningful.

The meaningful difference in performance can also be questioned in the study from Canivet and colleagues (2015). Their findings demonstrated the opposite pattern of results in that, in a population of 205 older adults (Mean Age = 72.7 years) Val carriers benefit more from increased PA than Met carriers do. They reported active Val carriers performed significantly better in a measure of episodic memory than inactive Val carriers, with a small effect size of partial eta squared .03 and no significant effect of PA on Met carriers. Similar results for Val carriers were found by Thibeau et al. (2016), where increased levels of PA were significantly associated with higher scores in executive functioning which was not seen in for Met carriers. In a population of 577 (Mean Age = 70.5 years), the authors found that for each unit increase in physical activity for Val carriers there was a significant increase of .43 in their scores of executive function, while Met carriers did not show a significant improvement with each increase in activity affecting their scores by .13 units. This result does indicate that more active Val carriers may experience a meaningful improvement in their executive functioning. The functions that represent the mechanisms of cognitive performance, such as executive functioning, have been demonstrated to decline over the adult lifespan (Hedden & Gabrieli, 2001). In contrast to the previous three studies, Thibeau and colleagues (2016), conducted a longitudinal study over a nine-year period, which may have produced the more robust findings.

The results of our study, that found no main effects or interaction effects between BDNF Val66Met and PA on cognitive performance across a battery of neuropsychological tests, contradict the previous findings of the same gene and environment interaction study. However, three of these studies revealed significant performances but with small effect sizes, bringing into question the true meaningful

effect of the interaction on cognitive performance demonstrated (Canivet et al., 2015; Erickson et al., 2013 & Kim et al., 2011). The fourth study, from Thibeau et al. (2016) demonstrated a significant, moderate positive effect of PA on cognitive performance for Val carriers over time but methodological differences make it difficult to compare the results of this interaction study with ours. Their population was older, with a mean age of 70.5 years and assessed decline over time while the age of our participants younger with a mean age of 64.2 years and assessed only one performance. It is possible that while there is no difference in the cognitive performance between genotypes and PA activity in our population, a significant variation in performance may occur in an older population.

Limitations

The younger mean age of the participants in our study may prove to be a limitation as it might have been too young to see the differences in performance between the BDNF Val66Met genotypes. Our findings confirmed the negative association between age and cognitive performance with three of the measures having a significant negetive correlation with participant age, however, sharper declines are observed over the age of 70 years (Hedden & Gabrieli, 2001). Levels of BDNF have also been shown to be negatively associated with age and the decline has been demonstrated to continue to occur at a significant level in older adults from 70 up to 103 years old (Zeigenhorn et al., 2007). This evidence, in combination with the association of the MET allele with lower availability of BDNF in the brain and lower brain volume, suggests it is possible that the negative impact for MET carriers on cognition won't be seen until much later in the life-span (Brown et al., 2014).

The IPEQ-WA, used for this study, has been shown to have good psychometric properties and internal consistency with the averaging of three-months

activities allowing for the week-to-week variability of more elderly participants (Doma, Speyer, Leicht & Cordier, 2017). However, use of self-report to determine PA level does have limitations. Participant's self-reports are a subjective measure that can result in a bias where they overinflate their estimates of activity frequency and duration (Shepherd, 2003). However, a systematic review and meta-analysis of 24 studies examining the relationship between physical activity and cognitive decline, across both questionnaire and objective measures, found 21 of the studies confirmed the positive association (Beydoun et al., 2014). This demonstrates that these varying measures are sensitive enough to establish the level of activity required to impact cognitive functioning. In addition, one of the particular benefits for self-report questionnaires in an aging population is the assessment of PA over various domains, such as gardening and housework, rather than the more rigid, objective measures that don't measure incidental activities (Warren et al., 2009). It is possible however that the use of an objective measure of physical activity, such as an accelerometer, might produce more robust effects and interactions with the BDNF Val66Met polymorphism than those observed in this study.

Another limitation may be volunteer/participation bias which is systematic error due to differences between those who chose to participate in studies and those who do not. Participation bias has been examined in a study of 1000 older adults by comparing those who volunteered to participate in an exercise study and those who did not (de Souto Barreto, Ferrandez & Salina-Serre, 2013). The researchers found that participants who were willing to take part in an exercise study were younger, had less physical function decline and higher levels of PA in comparison to those who declined to participate. Our demographic data revealed a breakdown of 25% (n = 50) in the Low PA group and 75% (n = 151) in the High PA group possibly

reflecting a bias in our population towards more physically active people. The effect of unequal sample sizes is a reduction in the power of the analysis to be able to detect a significant difference between the groups (Field, 2013). It is therefore reasonable to conclude that an increase in the number of less active participants would result in a more powerful study more able to detect a significant effect. Implementing stratified sampling to ensure a more equal representation between PA groups would address this.

Sample size is a considerable concern for studies examining the field of single nucleotide polymorphisms (SNP) such as BDNF Val66Met. In order to predict power, an estimate of effect size is used which is guided by the evidence in the literature and, as outlined previously the evidence for this gene x environment interaction study is minimal and inconsistent, making estimating effect sizes challenging. In a review of genetic research Payton (2006) suggests that replication for studies of SNPs with cognitive associations require around 1000 participants to achieve 80% power and our sample size of 201 participants may have lacked the power to observe a significant interaction between the groups. In fact, in a review of candidate gene x environment ($_{C}$G x E) interaction research, Duncan & Kelly (2011) found that only 27% of replication attempts found significant effects and that a publication bias existed where predominately novel, significant $_{C}$G x E studies are reported. The authors conclude that this bias reporting results in the literature reflecting a more robust pattern of effects than actually exist in $_{C}$G x E studies and suggest that the majority of the significant findings represent Type I errors. In conjunction with this information, it is possible the small effect sizes reported in the studies above are an indication of Type I errors and there was not a true significant difference between the groups cognitive performances.

Future Research

Due to the mixed findings to date, there is no discernable pattern of evidence yet, that can clearly define the relationship between the BDNF Val66Met polymorphism, level of PA and cognitive outcomes. It is possible that the variation in studies is due to interactions with other unexplored variables that future research should examine.

Although the interaction studies have exhibited conflicting results, the BDNF Val66Met polymorphism has become a very consistent candidate gene in cognition research (Mandelman & Grigorenko, 2012). In a review of candidate gene x environment interaction research, Dick and colleagues (2015) suggest that in order to account for a greater amount of the variance in the dependent variables it will be necessary for future research to examine genetic haplotypes. A haplotype is a combination of alleles or set of SNPs that tend to be inherited together (Jacobsson et al., 2008). There are very few studies that have examined the potential collusive effect on cognition of a haplotype including BDNF that could explain why the results of single genetic association studies have been so inconsistent.

As has been demonstrated with the BDNF Met allele, the APOE ε4 allele is associated with reduced cognitive function in an aging population with inconsistent results (Papenberg, Lindenberger & Bäckman, 2015). In one study, Ward et al. (2014), examined the combined influence of genetic polymorphisms, BDNF Val66Met and apolipoprotein E (APOE) on episodic memory function. Ward and colleagues (2014) found that while there was no significant difference for the independent genotypes on cognitive performance, there was a significant gene-gene interaction on episodic memory and the combination of the two risk alleles, the BDNF Met allele and the APOE ε4, resulted in the lowest mean performance.

Similarly, another research project examined the synergistic associations of BDNF, APOE and the genetic polymorphism of an additional gene implicated in healthy cognitive aging, catechol-O-methyltransferase (COMT) (Sapkota, Vergote, Westaway & Jhamandas, 2015). Sapkota and colleagues (2015) demonstrated an additive effect, whereby the combination of all three genetic risk alleles resulted in significantly reduced executive functioning performance that was not observed in the alternative combinations. It is therefore plausible that is may be the combination of genes, operating in an additive or synergistic way, that account for more of the variability demonstrated in cognitive performance with aging.

In addition, BDNF is not the only gene that had been associated with the effects of physical activity on cognitive outcomes. The interaction study discussed previously from Thibeau and colleagues (2016) also investigated the variation in the IDE (insulin degrading enzyme) gene and found a relationship between IDE, physical activity and executive functioning. Their results showed that carriers of the IDE G allele (considered to be the protective allele) that participated in higher levels of PA had significantly higher scores and less decline over time than the less active IDE G carriers. Increased amounts of physical activity in later life have also been demonstrated to mitigate the cognitive deficits associated with the APOE e4 allele (Luck et al., 2014). Also, in a study from Liang et al. (2010), the genetic susceptibility of increased amyloid deposition (a biomarker for Alzheimer's disease) in APOE e4 carriers was demonstrated to be reduced in participants who were more physically active. It is therefore evident that the variability seen in the cognitive benefits demonstrated by increased PA could be the result of multiple genetic influences.

As well as the inclusion of further genetic variables, cognitive performance can be modified by lifestyle factors in addition to PA. It is possible that an effect may be found when examining the relationship between the BDNF Val66Met polymorphism, cognitive function and other influential and interacting health factors. For example, dietary influences have been demonstrated to impact cognitive function with aging with a review from Parrott and Greenwood (2007) concluding that there is evidence that maximizing consumption of plant matter and healthy oils while limited saturated fats positively influences age-related cognitive changes. Taken together, all of this evidence suggests that future studies seeking to investigate the variability of cognitive decline associated with aging should focus on examining environment variables and their interactions with the synergistic effects of genetic haplotypes.

Finally, future studies need to employ different methodologies in order to assess the effect of the BDNF Val66Met polymorphism and level of PA on the decline associated with cognitive aging. Previous research has demonstrated the negative impact of the Met allele on cognitive performance in an older adult population over a number of years (Saptoka et al., 2017; Thibeau et al., 2016). These results imply that it is possible the negative effect of the Met allele confers its cognitive disadvantage over time and in order to find a significant difference between groups a longitudinal study would be more appropriate. Additionally, implementing a randomized controlled trial approach with an exercise intervention would enable a causal link to be drawn between the interaction of PA and BDNF genotype on cognitive outcomes if a significant effect is found.

Conclusion

This research study sought to examine the interactive effects of the BDNF Val66Met polymorphism and level of PA on cognitive performance in a healthy older adult population. While previous evidence has demonstrated the role of both BDNF genotype and PA independently and interactively in cognitive outcomes, our findings found no significant differences between the groups in measures of executive functioning, short-term memory, long-term memory or learning. The results of this study conclude that the BDNF Val66Met polymorphism and PA have no effect on the cognitive performance of healthy older adults. Limitations included our subjective self-report measure of PA, uneven distribution of Low PA and High PA participants and the age of our population. Future studies should consider using randomized controlled trials with objective measures of activity and take into account that it is possible that the effect on cognitive performance might not be evident until after the age of 70 years when the decline associated with normal cognitive aging occurs more rapidly. Additionally, examining genome wide genetic associations and the additive and synergistic relationships of haplotypes are also important considerations in the future of research into the variability of normal age-related cognitive decline.

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Appendix A

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HUMAN RESEARCH ETHICS COMMITTEE (TASMANIA) NETWORK

UTAS

31 July 2017

Ms Christine Padgett
C/- University of Tasmania

Sent via email

Dear Ms Padgett

REF NO: H0016623
TITLE: Exploring the Roles of Physical Activity and Genetic Predictors on Cognition in Older Adults

Document	***Version***	***Date***
Low risk Application		13 July 2017
HLAQ Modified for Australia		
Introduction for THBP Newsletter		
Cover letter Padgett		10 July 2017
Email and Mail introduction PA and Gene Study		
Finance and Administration		
Incidental and Planned Exercise Questionnaire		
PICF PA		13 July 2017

The Tasmanian Health and Medical Human Research Ethics Committee considered and approved the above documentation on **27 July 2017** to be conducted at the following site(s):

Wicking Dementia Research and Education Centre

Please ensure that all investigators involved with this project have cited the approved versions of the documents listed within this letter and use only these versions in conducting this research project.

This approval constitutes ethical clearance by the Health and Medical HREC. The decision and authority to commence the associated research may be dependent on factors beyond the remit of the ethics review process. For example, your research may need ethics clearance from other organisations or review by your research governance coordinator or Head of Department. It is your responsibility to find out if the approvals of other bodies or authorities are required. It is recommended that the proposed research should not commence until you have satisfied these requirements.

All committees operating under the Human Research Ethics Committee (Tasmania) Network are registered and required to comply with the *National Statement on the Ethical Conduct in Human Research* (NHMRC 2007 updated 2014).

Therefore, the Chief Investigator's responsibility is to ensure that:

(1) The individual researcher's protocol complies with the HREC approved protocol.

(2) Modifications to the protocol do not proceed until **approval** is obtained in writing from the HREC. Please note that all requests for changes to approved documents must include a version number and date when submitted for review by the HREC.

(3) Section 5.5.3 of the National Statement states:

Researchers have a significant responsibility in monitoring approved research as they are in the best position to observe any adverse events or unexpected outcomes. They should report such events or outcomes promptly to the relevant institution/s and ethical review body/ies and take prompt steps to deal with any unexpected risks.

The appropriate forms for reporting such events in relation to clinical and non-clinical trials and innovations can be located at the website below. All adverse events must be reported regardless of whether or not the event, in your opinion, is a direct effect of the therapeutic goods being tested. http://www.utas.edu.au/research-admin/research-integrity-and-ethics-unit-rieu/human-ethics/human-research-ethics-review-process/health-and-medical-hrec/managing-your-approved-project

(4) All research participants must be provided with the current Patient Information Sheet and Consent Form, unless otherwise approved by the Committee.

(5) The Committee is notified if any investigators are added to, or cease involvement with, the project.

(6) This study has approval for four years contingent upon annual review. A *Progress Report* is to be provided on the anniversary date of your approval. Your first report is due 27 July 2018. You will be sent a courtesy reminder closer to this due date.

(7) A *Final Report* and a copy of the published material, either in full or abstract, must be provided at the end of the project.

Should you have any queries please do not hesitate to contact me on (03) 6226 6254.

Yours sincerely

Jude Vienna-Hallam
Ethics Administration Officer

Appendix B

Participant Information Sheet [1.1] [7th August 2017]

UNIVERSITY of TASMANIA

PARTICIPANT INFORMATION SHEET

Exploring the Roles of Physical Activity and Genetic Predictors on Cognition in Older Adults

Invitation

We would like to invite you to participate in a research project investigating whether physical activity influences the way genes might impact cognitive function (for example memory and learning). This study is being run as a side-project by researchers in the Tasmanian Healthy Brain Project and researchers at the School of Medicine (Psychology).

The study is being conducted by:

- Dr Christine Padgett, Lecturer in the School of Medicine (Psychology), UTAS
- Associate Professor Mathew Summers, Associate Professor of Neuropsychology and Mental Health, Thompson Institute, USC and Investigator in the Tasmanian Healthy Brain Project, UTAS
- Professor James Vickers, Professor of Pathology, Wicking Centre, UTAS
- Kimberley Stuart, Research Fellow and Project Co-ordinator, The Tasmanian Healthy Brain Project UTAS
- Ruby Marris-Smith, 4th year Honours Student in the School of Medicine (Psychology) UTAS
- Melissa Heather, 4th year Honours Student in the School of Medicine (Psychology) UTAS

1. "What is the purpose of this study?"

There is evidence that some genes might influence cognitive function in later life (for example, memory and learning). However, it is possible that physical activity influences the effect of these genes. Therefore we would like to investigate the relationship between physical activity, genes thought to influence cognition, and cognition in older adults.

2. "Why have I been invited to participate in this study?"

You have been invited to participate in this study because you are currently participating in the Tasmanian Healthy Brain Project.

3. "What does this study involve?"

For this study, you would be asked to complete two short questionnaires; one asking you about past levels of physical activity and the other asking about your current levels of physical activity. These questionnaires can be either completed online or can be mailed to your home, where you can complete and the return in a postage-paid envelope that we will provide. Completing the questionnaires should only take about 10 minutes in total.

We would also ask that we could access the results of the cognitive assessments you have undertaken as part of the Tasmanian Healthy Brain Project, as well as the genetic results from the samples you provided. It is important to note that any data provided by researchers at the Tasmanian Healthy Brain Project would not have your name or any information that could identify who you are. Only Associate Professor Summers or Ms Stuart, who are also researchers on the Tasmanian Healthy Brain Project, would have your identifying information, and would remove these details and insert an alpha-numeric code in its place before passing on as to the current project.

4. "Are there any possible benefits from participating in this study?"

It is not expected that there will be any specific benefits from participating in this study.

5. "Are there any possible risks from participation in this study?"

We do not foresee any risks associated with participating in this study.

6. "What if I have questions about this research?"

If you would like to discuss anything about this study you are very welcome to contact Dr Christine Padgett on 6430 4946 or email her at Christine.Padgett@utas.edu.au. If you have concerns or complaints about the conduct of this study should contact the Executive Officer of the HREC (Tasmania) Network on (03) 6226 7479 or email human.ethics@utas.edu.au. The Executive Officer is the person nominated to receive complaints from research participants. You will need to quote HREC project number H0016623

Thank you for taking the time to consider this study. If you would like to take part, and have received this via email, please click on the link in this email. This will take you to a consent form and then on to the survey. If you have received this via mail, please complete the enclosed consent form and enclosed questionnaires and return using the postage paid envelope.

This information sheet is for you to keep.

UNIVERSITY of TASMANIA

CONSENT FORM

Exploring the Roles of Physical Activity and Genetic Predictors on Cognition in Older Adults

1. I have read and understood the information sheet for this project.
2. I understand that I will be asked questions relating to past and present physical activity, and that this survey will take approximately 10-15 minutes to complete.
3. I consent that the Tasmanian Healthy Brain Project can release my data to be included in this study.
4. I understand that there are no foreseen risks associated with this study.
5. I understand that all research data will be securely stored at the University of Tasmania for at least five years following publication of results, and will be destroyed when no longer required.
6. Any questions that I have asked have been answered to my satisfaction.
7. I agree that research data gathered from me for the study may be published provided that I cannot be identified as a participant.
8. I understand that any information I provide will be only used for the purposes of this research.
9. I understand that I may withdraw at any time without any consequences, and that I can request for my data to be removed from the study at any time.

If you have read and understood the information sheet and above points, and wish to be involved in the study, please click 'yes' below and you will be directed to the survey. If you do not wish to be part of this study, please click on the 'No' below and you will be exited from the survey. We thank you for your time.

Participant Consent Form [Mail-out 1] [June 2017]

UNIVERSITY of TASMANIA

CONSENT FORM

Exploring the Roles of Physical Activity and Genetic Predictors on Cognition in Older Adults

1. I have read and understood the information sheet for this project.
2. I understand that I will be asked questions relating to past and present physical activity, and that this survey will take approximately 10-15 minutes to complete.
3. I consent that the Tasmanian Healthy Brain Project can release my data to be included in this study.
4. I understand that there are no foreseen risks associated with this study.
5. I understand that all research data will be securely stored at the University of Tasmania for at least five years following publication of results, and will be destroyed when no longer required.
6. Any questions that I have asked have been answered to my satisfaction.
7. I agree that research data gathered from me for the study may be published provided that I cannot be identified as a participant.
8. I understand that any information I provide will be only used for the purposes of this research.
9. I understand that I may withdraw at any time without any consequences, and that I can request for my data to be removed from the study at any time.

Name of Participant:_____

Signature:_____ Date:_____

Appendix C.

Demographic Data and Analysis of Differences between Levels of Physical Activity (PA)

	Low PA (n = 50)	High PA (n = 151)	t-test/χ^2	*p* value
Age (years) Mean (SD)	64.28 (6.64)	64.18 (6.65)	.093	.926
Sex				
Males	14 (28.0%)	47 (31.1%)	.174	.677
Females	36 (72.0%)	104 (68.9%)		
BMI				
Mean (SD)	28.77 (6.06)	26.63 (10.31)	1.377	.170
HADS Scores				
Anxiety Mean (SD)	5.78 (3.62)	4.11 (2.97)	3.250	.001
Depression Mean (SD)	3.06 (2.56)	1.70 (2.09)	3.75	<.001
WTAR FSIQ				
Mean (SD)	112.76 (6.05)	112.64 (5.42)	.136	.892
Total Years of Education	15.58 (3.23)	16.56 (3.13)	-1.910	.058
Months since Cognitive Tests	17.84 (7.44)	17.44 (7.28)	.332	.740
THBP Experimental Group				
University Study	44 (88.0%)	115 (76.2%)	3.186	.074
Controls	6 (3.0%)	36 (23.8%)		
BDNF Genotype				
Met Carrier	25 (50.0%)	52 (34.4%)	3.85	.050
Val Carrier	25 (50.0%)	99 (65.6%)		

Appendix D.

Analysis of Covariance (ANCOVA) Between Genotype and Physical Activity Level on Cognitive Performance Measures with Age as a Covariate

		df	*F*	*p*	Cohen's *d*
Executive Functioning					
TMT B[a]	Genotype	1,194	1.40	.24	.07
	PA Level	1,194	0.56	.46	.001
	Interaction	1,194	1.42	.24	.009
Stroop[b]	Genotype	1,194	0.05	.83	.09
	PA Level	1,194	0.60	.44	.04
	Interaction	1,194	0.001	.97	.04
COWAT	Genotype	1,196	0.50	.48	.14
	PA Level	1,196	0.02	.89	.006
	Interaction	1,196	0.13	.72	.02
Short-term Memory					
Digit Span	Genotype	1,197	0.05	.83	.12
	PA Level	1,197	0.00	.99	.03
	Interaction	1,197	1.01	.32	.02
LNS	Genotype	1,196	0.10	.75	.004
	PA Level	1,196	0.001	.97	.004
	Interaction	1,196	0.24	.63	.004
Long-term Memory and Learning					
RAVLT[a]	Genotype	1,195	0.84	.36	.16
	PA Level	1,195	0.37	.55	.20
	Interaction	1,195	0.09	.76	.02
RCFT	Genotype	1,196	0.29	.59	.003
	PA Level	1,196	0.16	.69	.05
	Interaction	1,196	0.93	.34	.002

Note: Significance at $\alpha = .05$. [a] = HADS depression score was included as a covariate in the analysis. [b] = HADS anxiety score was included as a covariate in the analysis.